A

03/19/98 582 U.S. PTO

Practitioner's Docket No. 64675-004 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

Inventor(s): John P. Peeters, Ph.D.

WARNING: 37 C.F.R. § 1.41(a)(1) points out:

"(a) A patent is applied for in the name or names of the actual inventor or inventors.

"(1) The inventorship of a nonprovisional application is that inventorship set forth in the oath or declaration as prescribed by § 1.63, except as provided for in § 1.53(d)(4) and § 1.63(d). If an oath or declaration as prescribed by § 1.63 is not filed during the pendency of a nonprovisional application, the inventorship is that inventorship set forth in the application papers filed pursuant to § 1.53(b), unless a petition under this paragraph accompanied by the fee set forth in § 1.17(i) is filed supplying or changing the name or names of the inventor or inventors."

For (title):

NANOELECTRODE ARRAYS

CERTIFICATION UNDER 37 C.F.R. 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this New Application Transmittal and the documents referred to as attached therein are being deposited with the United States Postal Service on this date March 18, 1998, in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EI044902471US addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Donna Crumit
(type or print name of person mailing paper)

Donna Crumit
Signature of person mailing paper

WARNING: Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

WARNING: Each paper or fee filed by "Express Mail" **must have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

1. Type of Application

This new application is for a(n)

(check one applicable item below)

☒ Original (nonprovisional)

☐ Design

☐ Plant

WARNING: *Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4), unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

☐ Divisional.

☐ Continuation.

☐ Continuation-in-part (C-I-P).

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: A nonprovisional application may claim an invention disclosed in one or more prior filed copending nonprovisional applications or copending international applications designating the United States of America. In order for a nonprovisional application to claim the benefit of a prior filed copending nonprovisional application or copending international application designating the United States of America, each prior application must name as an inventor at least one inventor named in the later filed nonprovisional application and disclose the named inventor's invention claimed in at least one claim of the later filed nonprovisional application in the manner provided by the first paragraph of 35 U.S.C. 112. Each prior application must also be:

(i) An international application entitled to a filing date in accordance with PCT Article 11 and designating the United States of America; or

(ii) Complete as set forth in § 1.51(b); or

(iii) Entitled to a filing date as set forth in § 1.53(b) or § 1.53(d) and include the basic filing fee set forth in § 1.16; or

(iv) Entitled to a filing date as set forth in § 1.53(b) and have paid therein the processing and retention fee set forth in § 1.21(l) within the time period set forth in § 1.53(f).

37 C.F.R. § 1.78(a)(1).

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20–year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c–i–p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

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*WARNING: When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional application **must** be filed prior to the Saturday, Sunday, or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3)*

☐ The new application being transmitted claims the benefit of prior U.S. application(s). Enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

3. Papers Enclosed

A. Required for filing date under 37 C.F.R. § 1.53(b) (Regular) or 37 C.F.R. § 1.153 (Design) Application

_24_ Pages of specification

_6_ Pages of claims

_8_ Sheets of drawing

☐ formal

☒ informal

B. Other Papers Enclosed

_1_ Pages of Abstract

_____ Other

*WARNING: **DO NOT** submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. For comments on proposed then-new 37 CFR 1.84, see Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (5/8 inch) down from the top of the page . . ." 37 C.F.R. 1.84(c)).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)." 37 C.F.R. 1.84(b).

4. Additional papers enclosed

☐ Preliminary Amendment

☐ Information Disclosure Statement (37 C.F.R. 1.98)

☐ Form PTO–1449 (PTO/SB/08A and 08B)

☐ Citations

☐ Declaration of Biological Deposit

☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☒ Other - Small Entity Statement filed in U.S. Provisional App.

5. Declaration or oath

NOTE: A newly executed declaration is not required in a continuation or divisional application provided that the prior nonprovisional application contained a declaration as required, the application being filed is by all or fewer than all the inventors named in the prior application, there is no new matter in the application being filed, and a copy of the executed declaration filed in the prior application (showing the signature or an indication thereon that it was signed) is submitted. The copy must be accompanied by a statement requesting deletion of the names of person(s) who are not inventors of the application being filed. If the declaration in the prior application was filed under § 1.47, then a copy of that declaration must be filed accompanied by a copy of the decision granting § 1.47 status or, if a nonsigning person under § 1.47 has subsequently joined in a prior application, then a copy of the subsequently executed declaration must be filed. See 37 C.F.R. §§ 1.63(d).

☒ Enclosed

Executed by

(check all *applicable boxes)*

☒ inventor(s).

☐ legal representative of inventor(s).
37 CFR 1.42 or 1.43.

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. *See* item 13 below for fee.

☐ Not Enclosed.

NOTE: Where the filing is a completion in the U.S. of an International Application or where the completion of the U.S. application contains subject matter in addition to the International Application, the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.

☐ Application is made by a person authorized under 37 C.F.R. 1.41(c) on behalf of *all* the above named inventor(s).

(The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

☐ Showing that the filing is authorized.
(not required unless called into question. 37 CFR 1.41(d))

6. Inventorship Statement

WARNING: If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.

The inventorship for all the claims in this application are:

☒ The same.

or

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

☐ is submitted.

☐ will be submitted.

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. An English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application, or within such time as may be set by the Office. 37 CFR 1.52(d).

☒ English

☐ Non-English

☐ The attached translation includes a statement that the translation is accurate. 37 C.F.R. 1.52(d).

8. Assignment

☐ An assignment of the invention to ______________________________

____

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☐ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters-one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

WARNING: *A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993, 1150 O.G. 62-64.*

9. Certified Copy

Certified copy(ies) of application(s)

Country	Appln. No.	Filed
Country	Appln. No.	Filed
Country	Appln. No.	Filed

from which priority is claimed

☐ is (are) attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application, then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 C.F.R. 1.16)

A. ☒ Regular application

CLAIMS AS FILED					
	Number filed	Number Extra		Rate	Basic Fee 37 C.F.R. 1.16(a) $790.00
Total Claims (37 CFR 1.16(c))	37 − 20 =	17	×	$ 22.00	374.00
Independent Claims (37 CFR 1.16(b))	5 − 3 =	2	×	$ 82.00	164.00
Multiple dependent claim(s), if any (37 CFR 1.16(d))			+	$270.00	

☐ Amendment cancelling extra claims is enclosed.

☐ Amendment deleting multiple-dependencies is enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $ 1,328.00

B. ☐ Design application ($330.00—37 CFR 1.16(f))

Filing Fee Calculation $______________

C. ☐ Plant application ($540.00—37 CFR 1.16(g))

Filing fee calculation $______________

11. Small Entity Statement(s)

☐ Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is (are) attached.

WARNING: *"Status as a small entity must be specifically established in each application or patent in which the status is available and desired. Status as a small entity in one application or patent does not affect any other application or patent, including applications or patents which are directly or indirectly dependent upon the application or patent in which the status has been established. The refiling of an application under § 1.53 as a continuation, division, or continuation-in-part (including a continued prosecution application under § 1.53(d)), or the filing of a reissue application requires a new determination as to continued entitlement to small entity status for the continuing or reissue application. A nonprovisional application claiming benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) of a prior application, or a reissue application may rely on a statement filed in the prior application or in the patent if the nonprovisional application or the reissue application includes a reference to the statement in the prior application or in the patent or includes a copy of the statement in the prior application or in the patent and status as a small entity is still proper and desired. The payment of the small entity basic statutory filing fee will be treated as such a reference for purposes of this section." 37 C.F.R. § 1.28(a)(2).*

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(complete the following, if applicable)

☒ Status as a small entity was claimed in prior application 60 / 065,373, filed on 11/12/97, from which benefit is being claimed for this application under:

35 U.S.C. ☒ 119(e),
☐ 120,
☐ 121,
☐ 365(c),

and which status as a small entity is still proper and desired.

☒ A copy of the statement in the prior application is included.

Filing Fee Calculation (50% of **A, B** or **C** above)

$ 664 00

NOTE: *Any excess of the full fee paid will be refunded if small entitiy status is established and a refund request are filed within 2 months of the date of timely payment of a full fee. The two-month period is not extendable under § 1.136. 37 CFR 1.28(a).*

12. Request for International-Type Search (37 C.F.R. 1.104(d))

(complete, if applicable)

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made at This Time

☐ Not Enclosed

☐ No filing fee is to be paid at this time. *(This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.)*

☒ Enclosed

☒ Filing fee $ 664 00

☐ Recording assignment ($40.00; 37 C.F.R. 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION".) $ ______

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached ($130.00; 37 C.F.R. 1.47 and 1.17(i)) $ ______

☐ For processing an application with a specification in a non-English language ($130.00; 37 C.F.R. 1.52(d) and 1.17(k)) $ ______

☐ Processing and retention fee ($130.00; 37 C.F.R. 1.53(d) and 1.21(l)) $ ______

☐ Fee for international-type search report ($40.00; 37 C.F.R. 1.21(e)) $ ______

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application that is abandoned for failing to complete the application pursuant to 37 CFR 1.53(f) and this, as well as the changes to 37 CFR 1.53 and 1.78(a)(1), indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid, or the processing and retention fee of § 1.21(l) must be paid, within 1 year from notification under § 53(f).

Total fees enclosed $664.00

14. Method of Payment of Fees

☐ Check in the amount of $________

☒ Charge Account No. 04-2223 in the amount of $664.00.

A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should not be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☒ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 04-2223:

☒ 37 C.F.R. 1.16(a), (f) or (g) (filing fees)

☒ 37 C.F.R. 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☒ 37 C.F.R. 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☒ 37 C.F.R. §§ 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a)).

☒ 37 C.F.R. 1.17 (application processing fees)

NOTE: ". . .A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

☐ 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying, . . . issue fee." From the wording of 37 CFR 1.28(b), (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions as to Overpayment

NOTE: ". . . Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

- [x] Credit Account No. 04-2223
- [ ] Refund

SIGNATURE OF PRACTITIONER

Reg. No. 31,843

Robert L. Kelly
(type or print name of attorney)

Tel. No. (248) 203-0849

Dykema Gossett PLLC
P.O. Address

Customer No.

1577 N. Woodward, Suite 300

Bloomfield Hills MI 48304

☐ **Incorporation by reference of added pages**

(check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added ____________

☒ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added 6

☐ Plus added pages deleting names of inventor(s) named in prior application(s) who is/are no longer inventor(s) of the subject matter claimed in this application.

Number of pages added ____________

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added ____________

☐ **Statement Where No Further Pages Added**

(if no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item)

☐ This transmittal ends with this page.

(Application Transmittal **[4-1]**—page 10 of 10)

Practitioner's Docket No. 64675-004 ***PATENT***

ADDED PAGES FOR APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED

NOTE: See 37 CFR 1.78.

17. Relate Back

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

(complete the following, if applicable)

☒ Amend the specification by inserting, before the first line, the following sentence:

A. 35 U.S.C. 119(e)

NOTE: *"Any nonprovisional application claiming the benefit of one or more prior filed copending provisional applications must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior provisional application, identifying it as a provisional application, and including the provisional application number (consisting of series code and serial number)." 37 C.F.R. § 1.78(a)(4).*

☒ "This application claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	FILING DATE
60 / 065,373	11/12/97 "
____ / ____	____ "
____ / ____	____ "

20. Further Inventorship Statement Where Benefit of Prior Application(s) Claimed

(complete applicable item (a), (b) and/or (c) below)

(a) ☐ This application discloses and claims only subject matter disclosed in the prior application whose particulars are set out above and the inventor(s) in this application are

☐ the same.

☐ less than those named in the prior application. It is requested that the following inventor(s) identified for the prior application be deleted:

____

(type name(s) of inventor(s) to be deleted)

(b) ☐ This application discloses and claims additional disclosure by amendment and a new declaration or oath is being filed. With respect to the prior application, the inventor(s) in this application are

☐ the same.

☐ the following additional inventor(s) have been added:

____

(type name(s) of inventor(s) to be added)

(c) The inventorship for all the claims in this application are

☒ the same.

☐ not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made

☐ is submitted.

☐ will be submitted.

(Added Pages for Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed
[4-1.1]—page 4 of 5)

B. 35 U.S.C. 120, 121 and 365(c)

NOTE: "Except for a continued prosecution application filed under § 1.53(d), any nonprovisional application claiming the benefit of one or more prior filed copending nonprovisional applications or international applications designating the United States of America must contain or be amended to contain in the first sentence of the specification following the title a reference to each such prior application, identifying it by application number (consisting of the series code and serial number) or international application number and international filing date and indicating the relationship of the applications. . . . Cross-references to other related applications may be made when appropriate." (See § 1.14(a)). 37 C.F.R. § 1.78(a)(2).

☐ "This application is a

☐ continuation

☐ continuation-in-part

☐ divisional

of copending application(s)

☐ application number 0 /____________________ filed on ____________"

☐ International Application ____________________ filed on

____________________ and which designated the U.S."

NOTE: The proper reference to a prior filed PCT application that entered the U.S. national phase is the U.S. serial number and the filing date of the PCT application that designated the U.S.

NOTE: (1) Where the application being transmitted adds subject matter to the International Application, then the filing can be as a continuation-in-part or (2) if it is desired to do so for other reasons then the filing can be as a continuation.

NOTE: The deadline for entering the national phase in the U.S. for an international application was clarified in the Notice of April 28, 1987 (1079 O.G. 32 to 46) as follows:

"The Patent and Trademark Office considers the International application to be pending until the 22nd month from the priority date if the United States has been designated and no Demand for International Preliminary Examination has been filed prior to the expiration of the 19th month from the priority date and until the 32nd month from the priority date if a Demand for International Preliminary Examination which elected the United States of America has been filed prior to the expiration of the 19th month from the priority date, provided that a copy of the international application has been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively. If a copy of the international application has not been communicated to the Patent and Trademark Office within the 20 or 30 month period respectively, the international application becomes abandoned as to the United States 20 or 30 months from the priority date respectivley. These periods have been placed in the rules as paragraph (h) of § 1.494 and paragraph (i) of § 1.495. A continuing application under 35 U.S.C. 365(c) and 120 may be filed anytime during the pendency of the international application."

☐ "The nonprovisional application designated above, namely application ______ / ____________________, filed __________, claims the benefit of U.S. Provisional Application(s) No(s).:

APPLICATION NO(S).:	FILING DATE
______ /____________________	____________ "
______ /____________________	____________ "
______ /____________________	____________ "

☐ Where more than one reference is made above, please combine all references into one sentence.

(Added Pages for Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed **[4-1.1]**—page 2 of 5)

18. Relate Back—35 U.S.C. 119 Priority Claim for Prior Application

The prior U.S. application(s), including any prior International Application designating the U.S., identified above in item 17B, in turn itself claim(s) foreign priority(ies) as follows:

Country	Appln. no.	Filed on

The certified copy(ies) has (have)

☐ been filed on ________, in prior application 0 /____________, which was filed on ____________.

☐ is (are) attached.

WARNING: *The certified copy of the priority application that may have been communicated to the PTO by the International Bureau may **not** be relied on without any need to file a certified copy of the priority application **in the continuing application.** This is so because the certified copy of the priority application communicated by the International Bureau is placed in a folder and is not assigned a U.S. serial number unless the national stage is entered. Such folders are disposed of if the national stage is not entered. Therefore, such certified copies may not be available if needed later in the prosecution of a continuing application. An alternative would be to physically remove the priority documents from the folders and transfer them to the continuing application. The resources required to request transfer, retrieve the folders, make suitable record notations, transfer the certified copies, enter and make a record of such copies in the Continuing Application are substantial. Accordingly, the priority documents in folders of international applications that have not entered the national stage may not be relied on. Notice of April 28, 1987 (1079 O.G. 32 to 46).*

19. Maintenance of Copendency of Prior Application

NOTE: The PTO finds it useful if a copy of the petition filed in the prior application extending the term for response is filed with the papers constituting the filing of the continuation application. Notice of November 5, 1985 (1060 0.G. 27).

A. ☐ Extension of time in prior application

*(This item **must** be completed and the papers filed **in the prior application,** if the period set in the prior application has run.)*

☐ A petition, fee and response extends the term in the pending **prior** application until ____________________.

☐ A **copy** of the petition filed in prior application is attached.

B. ☐ Conditional Petition for Extension of Time in Prior Application

(complete this item, if previous item not applicable)

☐ A conditional petition for extension of time is being filed in the pending **prior** application.

☐ A **copy** of the conditional petition filed in the prior application is attached.

21. Abandonment of Prior Application *(if applicable)*

☐ Please abandon the prior application at a time while the prior application is pending, or when the petition for extension of time or to revive in that application is granted, and when this application is granted a filing date, so as to make this application copending with said prior application.

NOTE: *According to the Notice of May 13, 1983 (103, TMOG 6-7), the filing of a continuation or continuation-in-part application is a proper response with respect to a petition for extension of time or a petition to revive and should include the express abandonment of the prior application conditioned upon the granting of the petition and the granting of a filing date to the continuing application.*

22. Petition for Suspension of Prosecution for the Time Necessary to File an Amendment

WARNING: *"The claims of a new application may be finally rejected in the first Office action in those situations where (1) the new application is a continuing application of, or a substitute for, an earlier application, and (2) all the claims of the new application (a) are drawn to the same invention claimed in the earlier application, and (b) would have been properly finally rejected on the grounds of art of record in the next Office action if they had been entered in the earlier application." MPEP, § 706.07(b).*

NOTE: *Where it is possible that the claims on file will give rise to a first action final for this continuation application and for some reason an amendment cannot be filed promptly (e.g., experimental data is being gathered) it may be desirable to file a petition for suspension of prosecution for the time necessary.*

(check the next item, if applicable)

☐ There is provided herewith a Petition To Suspend Prosecution for the Time Necessary to File An Amendment (New Application Filed Concurrently)

23. Small Entity (37 CFR § 1.28(a))

☒ Applicant has established small entity status by the filing of a statement in parent application 60 / 065,373 on 11/12/97.

☒ A copy of the statement previously filed is included.

WARNING: *See 37 CFR § 1.28(a).*

24. NOTIFICATION IN PARENT APPLICATION OF THIS FILING

☐ A notification of the filing of this
(check one of the following)

☐ continuation

☐ continuation-in-part

☐ divisional

is being filed in the parent application, from which this application claims priority under 35 U.S.C. § 120.

Express Mail EI044902471US

Atty Docket
No. 64675-003
64675-004

PATENT

VERIFIED STATEMENT BY INVENTOR CLAIMING
SMALL ENTITY STATUS (37 C.F.R. 1.9(f) and 1.27(c))

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled:

NANOELECTRODE ARRAYS

described in the specification of United States provisional patent application filed concurrently herewith.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

NONE

I acknowledge the duty to file, in this provisional application or subsequent patent application, notification of any change in status result in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent to which this verified statement is directed.

Dated: 11/1/97

John P. Peeters, Ph.D.

136149

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: John P. Peeters

For: NANOELECTRODE ARRAYS

Attorney Docket No. 64,675-004

Assistant Commissioner For Patents
Washington, D.C. 20231

COMBINED DECLARATION AND POWER OF ATTORNEY
(Sole Inventor - No Priority Claimed)

As the below-named inventor, I hereby declare: that my residence, post office address and citizenship are as stated near my name below; that I believe I am the original and first inventor of the subject matter which is described and claimed in the specification of the above-captioned United States patent application and any amendment thereto submitted herewith (if any); that I have reviewed and understand the contents of the specification of this application, including the claims, as amended by any amendment referred to above;

I further declare: that as to the subject matter disclosed and claimed in this application, I do not know and do not believe the same was ever known or used in the United States of America before my invention thereof, or patented or described in any printed publication in any country before my invention thereof for more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application; that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representative or assigns more than twelve (12) months prior to this application; that I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations Section 1.56(a); and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns except as follows: NONE.

Docket No. 64,675-004

PATENT

I hereby claim the benefit under Title 35, United States Code, § 119(e) of United States provisional application Serial No. 60/065,373 filed November 12, 1997.

I hereby appoint Charles R. Rutherford, Registration No. 18,933; Robert L. Kelly, Registration No. 31,843; Kevin M. Hinman, Registration No. 35,193; John W. Rees, Registration No. 38,278; and Kevin G. Mierzwa, Registration No. 38,049, as my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith. Please address all correspondence and telephone calls to:

Robert L. Kelly
DYKEMA GOSSETT PLLC
1577 N. Woodward Avenue, Suite 300
Bloomfield Hills, Michigan 48304
(248) 203-0849

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of inventor: John R. Peeters

Inventor's Signature: ______________________

Post Office and Residence: 4607 HARLING LANE BETHESDA MD 20814

Citizenship: United States of America

Date: 3/16/1998

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Express Mail Label No.
EI044902471US

Attorney Docket No.
64675-004

PATENT

NANOELECTRODE ARRAYS

This application claims the benefit of U.S. Provisional Application No. 60/065,373 filed November 12, 1997.

TECHNOLOGICAL FIELD

The present invention relates generally to methods and apparatus for detecting and characterizing single biological molecules in solution and, more specifically, to detect and characterize individual proteins, protein mixtures, DNA or other molecules on a chip.

BACKGROUND OF THE INVENTION

The characterization and quantification of individual proteins or complex biological molecules is extremely important in fields as distant as medicine, forensics and the military. For example in medicine the presence and concentration of given proteins can be used for disease or pre-disease diagnoses. In the military given proteins can be used to signal the presence or absence of given pathogens in the environment which is extremely important for example in potential germ warfare situations.

The detection of individual proteins or molecules in biological samples is currently complex and generally requires sophisticated and bulky equipment.

Several technologies have recently been disclosed to characterize given biological molecules. In particular success has been achieved in high density DNA

chips build by Affymetrix as originally described in PCT International Publication No. WO 90/15070.

U.S. Patent 5,624,537, entitled "BIOSENSOR AND INTERFACE MEMBRANE", describes a protein-receiving matrix and a single electrode.

U.S. Patent 5,395,587, entitled "SURFACE PLASMON RESONANCE DETECTOR HAVING COLLECTOR FOR ELUTED LIGATE", describes a system to measure immobilized ligands using a plasmon resonance detector.

U.S. Patent 5,607,567 entitled "PROTAMINE-RESPONSIVE POLYMERIC MEMBRANE ELECTRODE", describes a membrane electrode.

U.S. Patent 5,328,847 entitled "THIN MEMBRANE SENSOR WITH BIOCHEMICAL SWITCH", describes a biosensor with a specific recognition biomolecule.

U.S. Patent 4,777,019 entitled "BIOSENSOR", describes a biosensor for biological monomers.

U.S. Patent 5,532,128, entitled "MULTI-SITE DETECTION APPARATUS", describes test wells combined with electrodes to detect given biological molecules.

U.S. Patent 4,983,510 entitled "ENZYMES IMMOBILIZED ON LATEX POLYMER PARTICLES FOR USE WITH AN AMINO ACID ELECTROSENSOR", describes an electrosensor with a latex polymer trap.

U.S. Patent 5,384,028 entitled "BIOSENSOR WITH A DATA MEMORY", describes a membrane biosensor with a memory module.

U.S. Patent 5,567,301 entitled "ANTIBODY COVALENTLY BOUND FILM IMMUNOBIOSENSOR", describes an antibody biosensor.

U.S. Patent 5,310,469 entitled "BIOSENSOR WITH A MEMBRANE CONTAINING BIOLOGICALLY ACTIVE MATERIAL", describes a membrane biosensor.

U.S. Patent 5,019,238 entitled "MEANS FOR QUANTITATIVE DETERMINATION OF ANALYTE IN LIQUIDS", describes a means to sequentially test the ionic concentration of fluids.

U.S. Patent 4,981,572 entitled "ELECTRODE UNIT AND PACKAGE FOR A BLOOD ANALYZER", describes an electrode and apparatus to analyze blood.

U.S. Patent 4,452,682 entitled "APPARATUS FOR MEASURING CLINICAL EMERGENCY CHECK ITEMS OF BLOOD", describes an apparatus to measure multiple elements in blood.

U.S. Patent 4,568,444 entitled "CHEMICAL SUBSTANCE MEASURING APPARATUS", describes an electrode to quantify chemical substances in a solution.

U.S. Patent 5,281,539 entitled "IMMUNOASSAY DEVICE FOR CONTINUOUS MONITORING", describes a two step immunoassay device.

U.S. Patent 5,192,507 entitled "RECEPTOR-BASED BIOSENSORS", describes a biosensor based on a polymeric film to detect opiates.

U.S. Patent 5,156,810 entitled "BIOSENSORS EMPLOYING ELECTRICAL, OPTICAL AND MECHANICAL SIGNALS", describes a thin layer biosensor.

U.S. Patent 5,494,831 entitled "ELECTROCHEMICAL IMMUNOSENSOR SYSTEM AND METHODS", describes an immunologic biosensor.

U.S. Patent 5,332,479 entitled "BIOSENSOR AND METHOD OF QUANTITATIVE ANALYSIS USING THE SAME", describes an electrode based sensor with a biologically active receptor.

U.S. Patent 5,582,697 entitled "BIOSENSOR, AND A METHOD AND A DEVICE FOR QUANTIFYING A SUBSTRATE IN A SAMPLE LIQUID USING THE SAME", describes a biosensor based on the measure of reduction between a substrate and an oxidoreductase.

U.S. Patent 4,908,112 entitled "SILICON SEMICONDUCTOR WAFER FOR ANALYZING MICRONIC BIOLOGICAL SAMPLES", describes a micro capillary separation device with detector capabilities.

U.S. Patent 5,409,583 entitled "METHOD FOR MEASURING CONCENTRATIONS OF SUBSTRATES IN A SAMPLE LIQUID BY USING A BIOSENSOR", describes a two step biosensor.

U.S. Statutory Invention H201 entitled "BIOSENSORS FROM MEMBRANE PROTEINS RECONSTITUTED IN POLYMERIZED LIPID BILAYERS", describes a method for incorporating and using cell membrane proteins in biosensors.

The above described technologies are generally used for the detection of a single type or a few different types of molecules. None of these technologies are particularly adapted to allow a very large number of different types of proteins, protein variants or other biological molecules to be detected and quantified simultaneously on a single chip. Furthermore none of the prior art provides a suitable technology to directly build protein-specific electronic receptors on a chip without the use of any biological binding agents, synthetic probes or complex micro-structures such as test wells.

We disclose herein a novel, smaller, faster and more cost effective technique to

detect, characterize and quantify individual proteins or other complex molecules on a chip. The technology described herein may also serve as a new method for DNA sequencing.

SUMMARY OF THE INVENTION

In one aspect the present invention provides a sensor which is capable of distinguishing between different molecular structures in a mixture. The device includes a substrate on which nanoscale binding sites in the form of multiple electrode clusters are fabricated. Each binding site includes nanometer scale points which extend above the surface of a substrate. These points are preferably nanoelectrodes which are spatially configured to provide a three-dimensional electro-chemical binding profile which mimics a chemical binding site. Thus, the binding sites have selective affinity for a complementary binding site on a target molecule or for the target molecule itself.

In one aspect, the binding sites are arranged in an array on the substrate. In one aspect, the spatial and electro-chemical profiles of each site of the array are identical and provide an assay for a single target molecule. In another aspect, regions of the nanoelectrode array carry grouped arrays of electronically and/or spatially distinct binding sites for simultaneous detection and quantification of several molecular species.

In still another aspect, the materials used for the electrodes and surrounding surfaces are selected based on preferred intrinsic electrical and chemical properties.

The nanoelectrode array may be included in a chamber which can retain fluids. Several arrays may be used in a single chamber and several different chambers may be used on a single chip.

In still another aspect, the nanoelectrode array and chamber are attached to at least one micro-fluidic delivery and separation system such as a micro-capillary which allows both the delivery and separation by size and electrical properties of the proteins or other molecules to be analyzed.

In another aspect a microcontroller or microprocessor is provided to analyze signals from the nanoelectrodes and/or to time and control the fluidics separation of the molecules or proteins.

In another aspect the chip with the nanoelectrode arrays is associated with an electronic temperature control system such as a thermoelectric device having a thermistor to vary the bonding kinetics or the electro-chemical affinity of the molecules with given nanoelectrodes, as well as the flow kinetics and separation of the molecules.

In another aspect the nanoelectrodes are interspaced in a linear microtube to sequence DNA.

Thus, it is an object of the present invention to provide a novel and rapid method

to analyze small biological molecules in solution such as proteins and to sequence DNA by using semiconductor chip technology with extremely high packing densities.

It is a further object of the present invention to ensure that the entire chip can be easily integrated into devices for automated analysis of proteins, DNA or other molecules.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective diagrammatic view of a nanoelectrode array showing different nanoelectrode clusters.

FIG. 2 is a side elevational diagrammatic view of a protein-specific electronic receptor and its matching protein.

FIG. 2A is a side elevational cross-section of a protein-specific electronic receptor and its matching protein.

FIG. 3 is a side elevational cross-section of a nanoelectrode array inside a micro-fluidic tube, showing the trapping of a specific protein on its corresponding nanoelectrode receptor.

FIG. 4 is a diagrammatic side elevational cross-section of a microtube with a

linear nanoelectrode array to detect DNA.

FIG. 5 is a cross-section of an integrated chip with nanoelectrode arrays, a micro-fluidic delivery system and associated electronics.

FIG. 6 is a side elevational cross-section of a nanoelectrode receptor showing the electrical field which is broken or modified upon binding of a specific molecule to said receptor.

FIG. 7 is a view of a cantilevered nanoplate with several identical nanoelectrode clusters.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is based in part on the fact that recent advances in technology such as the use of scanning tunneling microscopy (STM) has demonstrated that ultra small structures of a single or a few atomic layers can be built on a semiconductor surface such as silicon. Because of the size of these structures, they are generally referred to as nanostructures (one nanometer or nm = 10^{-9}m, 1 Angstrom or Å = 10^{-10}m). These structures can be as small as a few Angstroms in diameter which is well below the Stokes radius of a small protein (which is approximately 25-35Å). Since these structures can be built using different chemical elements (or the voltage applied to the structure can be selectively varied) and the spacial distribution, height

width and shape of the structures can also be varied, these structures can be built in clusters to serve specifically as "molecular electrodes" whose electro-chemical properties and spacial distribution can be made to correspond precisely with the external three dimensional shape and electro-chemical properties of molecules, preferably biochemicals and most preferably proteins. Therefore each of these clusters can serve as individual electronic protein "receptors" (or detectors). Since a very large number of these molecular electrodes can be placed on a single chip, the resulting arrays, termed here "nanoelectrode arrays" can be used to detect, characterize and quantify many different proteins on a single chip. In a variation of the technology, the chip can also be used to sequence DNA.

Referring now to Figure 1 of the drawings, microelectronic molecular sensor 20 is seen having substrate 22 on which an array of binding sites or clusters 24 are formed. Substrate 22 may comprise any number of materials such as silicon, germanium, gallium arsenide, or other semiconductors. Referring now to Figure 2 of the drawings, one binding site 24 is shown in more detail having multiple electrodes 26a, 26b and 26c which are spatially distributed to form a pattern. Thus, it can be seen that each electrode 26a, 26b and 26c in this particular embodiment is spaced laterally from the adjacent electrode and is elevated at different heights off principal surface 28 of substrate 22.

It will be appreciated that through molecular modeling and empirical data, the

topology of the binding sites and electrical charge are tailored to provide the required electrical and topographic properties to selectively recognize and bind a complementary region of a target molecule. As shown best in Figure 2, protein 30 having a defined shape specific to that protein attaches to a given nanoelectrode cluster composed of three nanoelectrodes 26a, 26b and 26c. As will be explained more fully, each nanoelectrode may have slightly different electro-chemical properties because of differing charges and/or chemical compositions. These individual electro-chemical properties match not only the electro-chemical affinities of the amino acids or atoms present on the grooves of the protein but also complement the shape of the groove itself. Thus, when a molecule having the proper complementary profile binds to "receptor" 24 bridging the gap between the electrodes, a change in electrical potential occurs which can be monitored through appropriate circuitry to provide an indication of the presence of the target molecule.

In the most preferred embodiments of the present invention binding sites 24 have nanoscale geometries. As illustrated in Figure 2, the distance from principal surface 28 to the top of electrode 26b is 1.9 nanometers, the width of electrode 26b is 0.7 nanometers and the distance between electrodes 26b and 26c is 1 nanometer. In general, each electrode will typically be between 0.2 and about 3 nanometers in height and from about 0.2 to about 2 nanometers in width. As used herein "nanoelectrode" shall include atomic scale as well as nanoscale structures, i.e. from 2Å to 5 nanometers. There will also typically be from about 2 to about 8 separate electrodes

in each cluster 24. Electrodes 26a, 26b and 26c can be formed of a number of materials, either intrinsic or doped, such as gold and platinum and copper and other electrometals. Gold is particularly preferred. Also it may be suitable to form the electrodes of one material and coat the outer portion with a different material, e.g. gold coated with zinc oxide or gold coated with a thiol group.

The electrodes may be each separately connected to a power source by small conductive regions or wires which may be formed of gold. In Figure 2A, individual conductive layers 34a, 34b and 34c are shown electrically connecting their respective electrodes 26a, 26b and 26c. Dielectric layers 36 electrically isolate the individual conductive layers and dielectric sheaths 38 electrically isolate the individual electrodes. It will be appreciated that different potentials can be applied to the various individual electrodes and that electrodes from different clusters can be electrically linked to a single layer e.g., layer 34a. It will be appreciated that the various layers can be formed using conventional thin-film fabrication techniques such as CVD, thermal growth and ion implantation.

It has been shown recently that electrical "wires" can be built of single atoms (see for example review by Leo Kouwenhoven "Single-Molecule Transistors", Science Vol. 275, pages 1896-1897, 28 March 1997, the entire disclosure of which is incorporated herein by reference). The wires can be deposited in a number of different ways as part of the microchip fabrication process, prior to the deposition of the

nanoelectrodes. The nanoelectrodes can be deposited directly on the chip by Scanning Tunneling Microscope (as described in Kolb et al., Science pages 1097-1099, Vol. 275, 21 February 1997, the entire disclosure of which is incorporated herein by reference). A number of other chip fabrication methods are possible such as different lithography techniques, etc.

In another aspect the nanoelectrodes are not connected to any electrical wires or conductive layers. In this case the binding of the protein or other molecule is simply dependant on the shape and chemical properties of the individual nanoelectrode clusters. Detection of the attachment of the given molecule to a given cluster can then be achieved by means other than electrical, for example by a highly precise x-y positional fluorescence reader, similar to that used for the DNA chip technology or by resonance.

In case the nanoelectrodes are not connected to wires (i.e. are not "live" electrodes), the nanoelectrodes may in some applications be interconnected in a given cluster. In this case the clusters would comprise interconnected peaks and grooves and these would form a larger structure (i.e. from 1 to >10 nanometers). This structure could be tailored either to match precisely the actual biological receptor of the target molecule or to allow the entire molecule to fit into a 3-dimensional "receptor" which would match at least a third of the overall 3-D shape of the molecule. In some instances and depending on the overall shape of the molecule, the receptor that is built

may not necessarily include a site corresponding to the actual biological receptor of the target molecule.

Several types of binding or adsorption of the molecule to the nanoelectrode receptor are possible, depending on the chemical composition of the nanoelectrodes, the voltage and the chemical to be measured. Binding forces may include covalent binding, electrostatic binding, hydrogen bonds and van der Waals bonds.

Depending on the type of detection that is required, the individual nanoelectrodes of individual clusters do not necessarily need to be composed of different electrometals since both the spacial distribution and the height of the nanoelectrodes can be varied and these two variables may be enough for specific molecule detection in given applications. In some applications, each nanoelectrode can be selectively charged in a given cluster, allowing the electro-physical property of the nanoelectrode to be varied.

The entire sensor can be built using a computer controlled operation, where the spacing, height, width and the composition of the nanoelectrodes can be made to correspond exactly to the three dimensional shape and matching electro-chemical properties of a selected molecule. Furthermore since the position of the nanoelectrode clusters corresponding to a given receptor for a given molecule is determined during the fabrication process, this position information can be used to detect attachment or

binding. For example a large nanoelectrode array can be built with many different clusters, binding in a solution can be allowed, then the array be read using a highly accurate x-y reader in a way similar to the DNA chip. Computer control fabrication of the nanoelectrodes also allows for identical copies of the chip to be made.

It will be also be appreciated that the geometries that are built on the surface of the chip can be made to correspond exactly to the matching image of a crystallized protein surface taken from x-ray diffraction studies. Hence nanoelectrode array clusters can be built directly using crystallographic data and the resulting surfaces on the chip would favor protein-specific crystallization on given arrays.

In another aspect since multiple identical receptors can be built on the same chip, this technology can be used not only to detect given molecules but also to precisely estimate the quantity of these molecules present in the sample by measuring binding rates in identical clusters.

Referring to FIG. 3, two partial nanoelectrode arrays are shown facing each other and forming micro-channel or nanotube 60, which permits the flow of small molecules such as protein 70 therethrough. If protein 70 matches the shape of a receptor composed of electrodes 74, 78, and 82, the physical binding of the protein will cause a temporary minute change in the electrical signal which can be measured simultaneously in all said nanoelectrodes. The strength of the electrical signal can be

modified for example by adding a conductant to the carrier solution for the molecules which need to be studied. Alternatively the nanoelectrodes themselves can be charged with a small current, which would change upon attachment of the given molecule. Depending on the electro-chemical properties of the nanoelectrodes and the analyte, the temperature and the flow rate, the binding may last only a fraction of a second or last longer. Time of retention in itself is another important variable which can be used in detecting and quantifying the types of molecules present in the sample.

In some applications, micro-channel 60 can form a part of a network of channels of different and specific sizes, matching the sizes of the proteins to measure. Each of these channels can be equipped with molecular sieves, allowing only proteins or molecules of certain size to pass through. The channels themselves can also serve as a means to separate molecules and deliver them to given detector chambers with nanoelectrode arrays which are specifically made to measure given classes of proteins or molecules of given molecular weights. In this case, each of the arrays would have nanoelectrodes with sizes corresponding to the sizes of the proteins to measure. As part of this network of channels, specific chambers can be added with specific functions such as a chamber to lyse cells. Other chambers can be filled with specific reagents which can be used as needed.

In another application each of the micro-channels is equipped with only one or a few nanoelectrode clusters and the protein mix is flowed through each of the

channels. With the help of a microcontroller or a microprocessor controlling the flow rate in each micro-channel, the signals from each of the nanoelectrode clusters is then measured combining the power of the following variables for detection: protein separation rates (based on the size and charge of the proteins) and retention time on each given cluster (based on the shape and electro-chemical properties of the molecule). Indeed the more a given molecule matches a given receptor, the longer it will bind. It is obvious that the sophisticated control and measure of the electrical signals in each nanoelectrode (as well as the control of all other variables such as sample flow rates, temperature, etc.) can only be done with the help of a microcontroller or a microprocessor.

Referring now to FIG. 4, a nanoarray of electrodes 90 is built in a linear microtube 100 with the spacing and electro-chemical composition of the nanoelectrodes varied in such a way to correspond exactly to the distance between given base pairs of a linear piece of DNA or RNA 110. In this case the nanoelectrodes are built using only two variables: precise spacing and electro-chemical composition (not height) favoring position-specific binding of specific base pairs of DNA or RNA to matching nanoelectrodes. The principle that is applied here is that DNA is known to behave as a linear molecule when flowed in a microtube and that this rate of flow can be controlled and measured with precision. Furthermore the distance between 10 DNA base pairs being precisely 34 Å, the nanoelectrodes can be spaced precisely in multiples of 3.4 Å as shown in 120. By varying the spacing and charge and/or composition of the

nanoelectrodes and by measuring the conductance changes over time in sequentially placed nanoelectrodes, an entire sequence is created, based on the timing of the signals of position-specific nanoelectrodes. The full DNA (or RNA) sequence is then reconstructed with the help of a microcontroller (or microprocessor) which can also control the flow rate in the microtube.

ANALYSIS OF PROTEIN VARIANTS

Mutations or other changes in the DNA result in amino-acid substitutions in the protein. These substitutions in turn result in conformational shape changes in the protein and can result in proteins that are either non-functional or have different properties. Since the three-dimensional (3-D) structure of proteins can now be inferred with precision on the basis of x-ray crystallography or nuclear magnetic resonance (NMR), the 3-D shapes of the protein variants can also be generated using the same method. Hence the entire spectrum of protein variants for given classes of proteins can be measured and quantified using the nanotechnology described above. This is because the conformational changes of each protein variant can be represented by a given nanoelectrode cluster varying in the shape, distribution and electro-chemical properties of the nanoelectrodes. In fact the building of the arrays can be computer-controlled and link the information matching the putative 3-D structure of proteins of interest (and their variants) to the micro fabrication of all the matching receptors on the chip. By measuring and quantifying these variants as described above, this approach represents a powerful alternative to direct DNA sequencing since all the possible

mutation products of given genes which are expressed can be directly measured on a chip. Another advantage is that the chip would be fully reusable. Furthermore given the extremely high density of the nanoelectrode arrays that can be built on a single chip, the entire spectra of protein variants for many genes can be measured at once on the same chip. In fact with a refinement in the technology, all existing human proteins and their variants could theoretically be measured on a single chip of 1 cm^2 and the number of receptors that could be built on such a chip could theoretically exceed 1 billion which is a thousand fold improvement over any existing technology.

PROTEIN SEPARATION

As indicated above, the separation of molecules can be achieved by flowing said molecules in extremely small tubes (micro-capillaries, micro-channels or nanotubes) where smaller molecules travel faster than larger ones which are retained by friction and weak bonding interactions with the surfaces of the tubes. The result that is achieved is equivalent to electrophoresis but with the advantage of speed, cost and reusability of the micro-capillary.

Referring now to FIG. 5, micro-channel 130 is shown with a sample input port 132 and a long loop flowing into an optional reagent micro-chamber 134, itself connected to an optional input port 136. Micro-channel 130 separates biological molecules by size and charge while micro-chamber 134 allows the selective input of an external reagent or solution. The flow and on/off position at each micro-channel

juncture can be controlled electronically either by an external micro-pump (not shown), by thermocapillary action or by a change of electric potential. After entering micro-chamber 134, the analyte then flows successfully into micro-chambers 138a, 138b, 138c, then 138d, each holding different nanoelectrode arrays with nanoelectrode clusters of varying sizes and densities. In this particular design, the nanoelectrode arrays are fabricated immediately adjacent to a micro-electronics multiplexing or control area 140, itself connected to an interface 142. After reacting with successive nanoelectrode arrays in successive micro-chambers, the sample exits via port 146. The micro-channels and micro-chambers can either be etched in the silicon surface itself or can be fabricated separately on a surface of a material like glass, diamond, plastic or the like, which is then attached to the silicon surface.

This design can be varied in many different ways and FIG. 5 illustrates just one of many possible combinations of micro-channels, nanoelectrode arrays and micro-electronics that can be fabricated on a chip. As indicated above, a chamber allowing the lysing of the cells or viruses to be analyzed can also be included on the chip. Also it should be indicated that the directional flow in the micro-channels can be reversed and that each connecting micro-channel can be selectively opened or closed electrically. Hence when the test is completed the entire system can be heated to allow protein denaturation (and/or the potential in the nanoelectrodes can be reversed), then the system can be flushed with a solution to clean the nanoelectrode arrays and allow reuse of the chip.

Hence a complete and integrated protein separation and detection system can be built on a single chip. An important aspect of combining nanoelectrode arrays, micro-channels and a microcontroller (or a microprocessor) is that the time of separation (from sample injection into port 132 to time of first detection) and the length of retention on given nanoelectrode receptors are important variables for characterizing individual protein or protein variants. For example, the system can be calibrated by injecting known proteins, then known mixes of proteins, prior to injecting the sample to be tested. The time taken to reach a given nanoelectrode receptor and the length of binding on different electronic receptors would be specific to specific proteins (or to protein variants) and the signal-specific profiles for each protein can then be stored in memory and compared to those of the sample to be tested.

While FIG. 5 shows an integrated design, it is obvious that the protein separation component and the electronic components can also be placed externally and that the chip can be as simple as having a single nanoelectrode array enclosed in a single chamber with an interface. This chip (which may be disposable) can then be inserted into a larger module with the above components. Also, as indicated below, other detection methods can be used and the design of the chip would change accordingly.

DETECTION

There are many ways in which the binding or adsorption of the analyte on the nanoelectrode array can be detected. Referring now to FIG. 6, one way of detecting

the signal due to adsorption on the nanoelectrode array is by electrical signal. In this case at least one of the electrodes in each cluster of a given array is used as a "source" 160 while the rest of the cluster 165 is used as a "sink." When an analyte, say a protein, is adsorbed it changes the flow of the current (pico ampere) as shown in FIG. 6. The electrodes are isolated by a layer of oxide 170. The unwanted effects of the electrical current can be avoided by using an AC approach.

Referring now to FIG. 7, the second approach for detection of binding is by using a resonance approach. In this method, a nano structure is constructed. For example, nanoplate 180 of the dimension less than one micron is built. This structure can be free standing or it can be cantilevered. Identical sets of nanoelectrode receptors 24 are then fabricated on this surface. The structure is designed to have resonance frequency in the MHz to low GHz region. As the analyte flows past these structures, they spend a longer time on the cantilever if they have a structure that is complementary to the nanoelectrode structure. In other words, the analyte molecules undergo collision with the nanoplate. If there exists any complimentary nature between the analyte and the substrate, the analyte will spend more time on the surface during collision. This can be detected optically by shining a laser diode on the structure and detecting the reflected signal using a position sensitive photodiode. The AC signal in the photodiode shows the resonance response of the structure. The greater the signal, the larger the concentration of bound biological molecules, i.e. the greater the concentration of the said molecule in the solution. Other detection techniques such as capacitive,

piezoresistive, piezoelectric, electron tunneling, etc. could also be used.

The structure can be excited into resonance response by mechanical means using a piezoelectric element. In this technique, a nanoplate structure is attached to a piezoelectric material which can be vibrated using an AC signal. At resonance the structures oscillate with maximum amplitude. It can also be excited into resonance by modulating the diode laser using square wave power pulses. Since square waves contain all the Fourier components, there will be a component that corresponds to the resonance frequency of the structure.

Since these nanoelectrodes can be constructed on geometrical structures with extremely small thermal mass (for example, nanoplates have a thermal mass of the order of many picograms or less), they can be heated and cooled in the micro to milliseconds time frame. This fact can be used to adsorb and desorb analytes in a periodic fashion. However, when there is a complimentary structure between surface and the analytes the desorption time scale will be different.

Use of an external detector

In another detection application the entire chip which has been allowed to react with the sample is placed in a x-y laser reader in a manner similar to the DNA chip. In this case the chip is incorporated into a highly precise holder to ensure accurate position reading of each cluster. Detection may be done by fluorescence, for example

after reaction of the bound samples to the clusters with a fluorescent molecule or with labeled antibodies.

Detection may also be done by other means such as laser-desorption-ionization mass spectrometry.

NANOELECTRODE CONSTRUCTION

Nanoelectrode arrays can be constructed on a doped semiconductor substrate by nanolithography using scanning probes. In this approach, metal clusters are deposited either from a solution or by field evaporation from a STM/AFM tip. Since the electric field between the tip and the substrate is very high (10^9V/m), many metals can be field evaporated. In solution many metals can be electrochemically deposited on a surface. The surface of the semiconductor can be oxidized to be an insulator.

Nanometer scale trenches and lines can be made on a semiconductor surface using STM tip in an oxide etching solution producing a trench. The depth of the trench depends on the time spent by the tip at that location and the voltage on the tip. Hence not only can the nanoelectrodes be built by deposition but they can also be built by etching. The trenches can also be used to make the channels to separate the proteins, as instructed above.

It should also be noted that nanotransistors can be built directly in the chip to

facilitate detection and increase the density of the detectors. The nanotransistors can be build prior to the deposition of the nanoelectrodes as a sub-layer in the overall chip manufacturing process or be placed on an adjacent part of the chip.

The above-described principles illustrate the wide variety of applications that are possible in the micro fabrication and applications of the nanoelectrode arrays. For example the entire system from sample input to detection with output signals sent to an external device such as a monitor can be built on a single chip, using micro-channels (for sample separation and delivery), miniature ionic pumps, sample detection, a built-in microcontroller, a method for temperature control, etc. This chip can be inserted into a measuring device, for example for use in a physician's office or into a field detector. If a very large nanosensor array is used, it may be preferable to use a microprocessor or several microcontrollers to control the above described functions. In some applications the large arrays can be used with an external laser reader. In this case the array can be used in a way similar to the DNA chip, where the entire chip is allowed to react with the entire sample, washed and then inserted into an external reader. Using this approach the chip can be build into a convenient handling cassette.

While the invention has been described with respect to specific embodiment for complete and clear disclosure, the appended claims are not to be thus limited but are to be construed as embodying all modifications and alternative constructions that may occur to one skilled in the art which fairly fall within the basic teaching here set forth.

CLAIMS

What is claimed is:

1. A sensor for detecting biological molecules, said sensor comprising:

a substrate;

an electrode having the capacity to bind a preselected biological molecule, said electrode being between about 10^{-9} and 10^{-10} meters in height and width.

2. The sensor recited in claim 1, wherein said electrode is a plurality of electrodes.

3. The sensor recited in claim 2, wherein each of said electrodes has an identical chemical composition.

4. The sensor recited in claim 2, wherein at least one of said electrodes has a chemical composition which is different than the other of said electrodes.

5. The sensor recited in claim 1, wherein said electrode has an outer coating.

6. The sensor recited in claim 2, wherein each of said electrodes has a chemical coating.

7. The sensor recited in claim 6, wherein each of said coatings has the same chemical composition.

8. The sensor recited in claim 6, wherein at least one of said coatings is different than the other of said coatings.

9. The sensor recited in claim 2, wherein the height of at least one of said electrodes is different that the height of the other of said electrodes.

10. The sensor recited in claim 2, wherein the width of at least one of said electrodes is different that the widths of said other electrodes.

11. The sensor recited in claim 2, wherein said electrodes are spaced laterally from one another on said substrate.

12. The sensor recited in claim 2, wherein said electrodes are arranged in clusters on said substrate.

13. The sensor recited in claim 2, wherein the electro-chemical properties, width and spacing of said electrodes complement and bind a site on said biological molecules.

14. The sensor recited in claim 1, wherein said electrode is connected to at least one electrically conductive nanowire.

15. The sensor recited in claim 2, wherein said electrodes are connected to nanowires.

16. The sensor recited in claim 1, further comprising an interface connecting said sensor to a control system.

17. The sensor recited in claim 12, wherein said clusters are spaced to form an array.

18. The sensor recited in claim 1, wherein said biological molecules are proteins.

19. A sensor for detecting proteins, said sensor comprising:

a micro-capillary tube;

a plurality of electrodes disposed in said tube, said electrodes having the capacity to bind a preselected protein, said electrodes being between about 10^{-9} and 10^{-10} meters in height and width.

20. The sensor recited in claim 19, further comprising a microcontroller.

21. The sensor recited in claim 20, further comprising a system to regulate the temperature of said sensor.

22. A sensor for detecting biological molecules, said sensor comprising:

a substrate;

a micro cantilever array on said substrate;

at least one electrode disposed on at least one of said micro cantilevers.

23. The sensor recited in claim 22, further comprising a laser for determining the concentration of biological molecules bound to said electrode.

24. The sensor recited in claim 23, further comprising a piezoelectric detector for detecting the concentration of biological molecules bound to said electrode.

25. A method of sequencing nucleic acids, comprising the steps of:

providing a sensor, said sensor having a substrate on which plurality of electrodes are disposed, said electrodes each being between about 10^{-9} and 10^{-10} meters in height and width;

contacting said electrodes with a solution containing nucleic acids;

said electrodes having the capacity to bind at least some of said nucleic acids.

26. The method recited in claim 25, wherein said nucleic acids are DNA and wherein said electrodes are spaced apart from one another to complement and bind to DNA base pairs of a linear DNA molecule.

27. The method recited in claim 25, wherein said sensor includes a microtube in which said electrodes are disposed.

28. The method recited in claim 27, further comprising a flow control system and a laser detector.

29. The method recited in claim 25, further comprising a microcontroller and a display.

30. The method recited in claim 25, wherein said nucleic acids are RNA.

31. The method recited in claim 2, further comprising a support structure for said substrate, said support structure being adapted to be received in a x-y fluorescent laser reader.

32. A silicon chip to detect individual proteins comprising at least one sensor manufactured with Angstrom level precision where the surface of the sensor complements exactly the three dimensional shape of a given protein.

33. The invention recited in claim 32, wherein the sensor is made of a single metal.

34. The invention recited in claim 32, wherein the sensor is made of different metals.

35. The invention recited in claim 32, wherein the sensor forms a protein-specific receptor.

36. The invention recited in claim 32, wherein the sensor is made from information derived from x-ray diffraction studies.

37. The invention recited in claim 32, wherein the sensor is made from information derived from nuclear magnetic resonance studies.

ABSTRACT

An array of electrodes at the atomic or nano scale (nanoelectrodes) is built on a chip. The spatial distribution, height, width and electro-chemical composition of the nanoelectrodes is varied, such that protein-specific electronic receptors are built directly on the chip with the nanoelectrodes without the use of any specific binding agents or molecules. Because of their size, a very large number of different receptors can be built as arrays on a single chip. The chip can be used to detect, characterize and quantify single molecules in solution such as individual proteins, complex protein mixtures, DNA or other molecules.

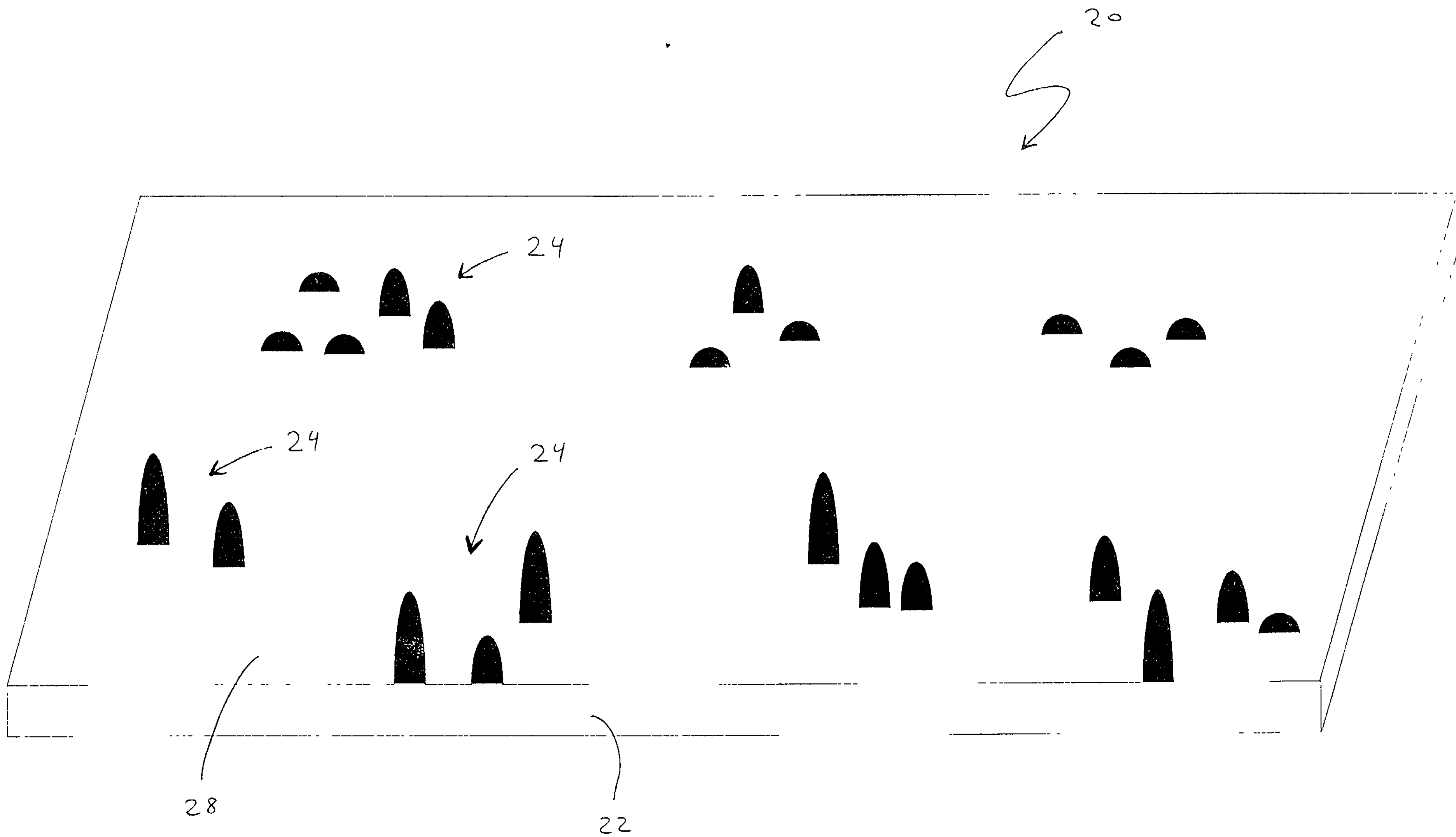

FIG. 1

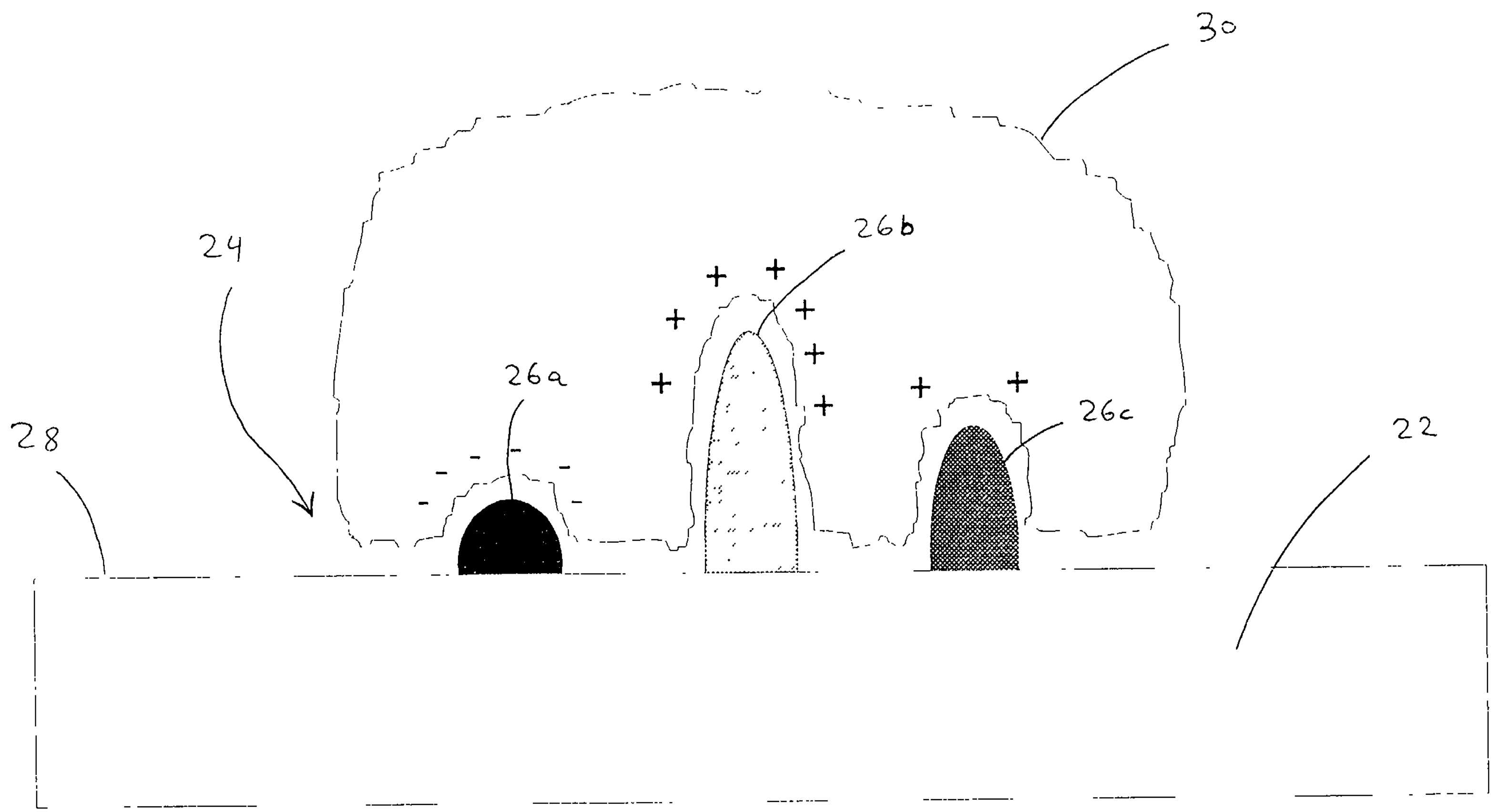

FIG. 2

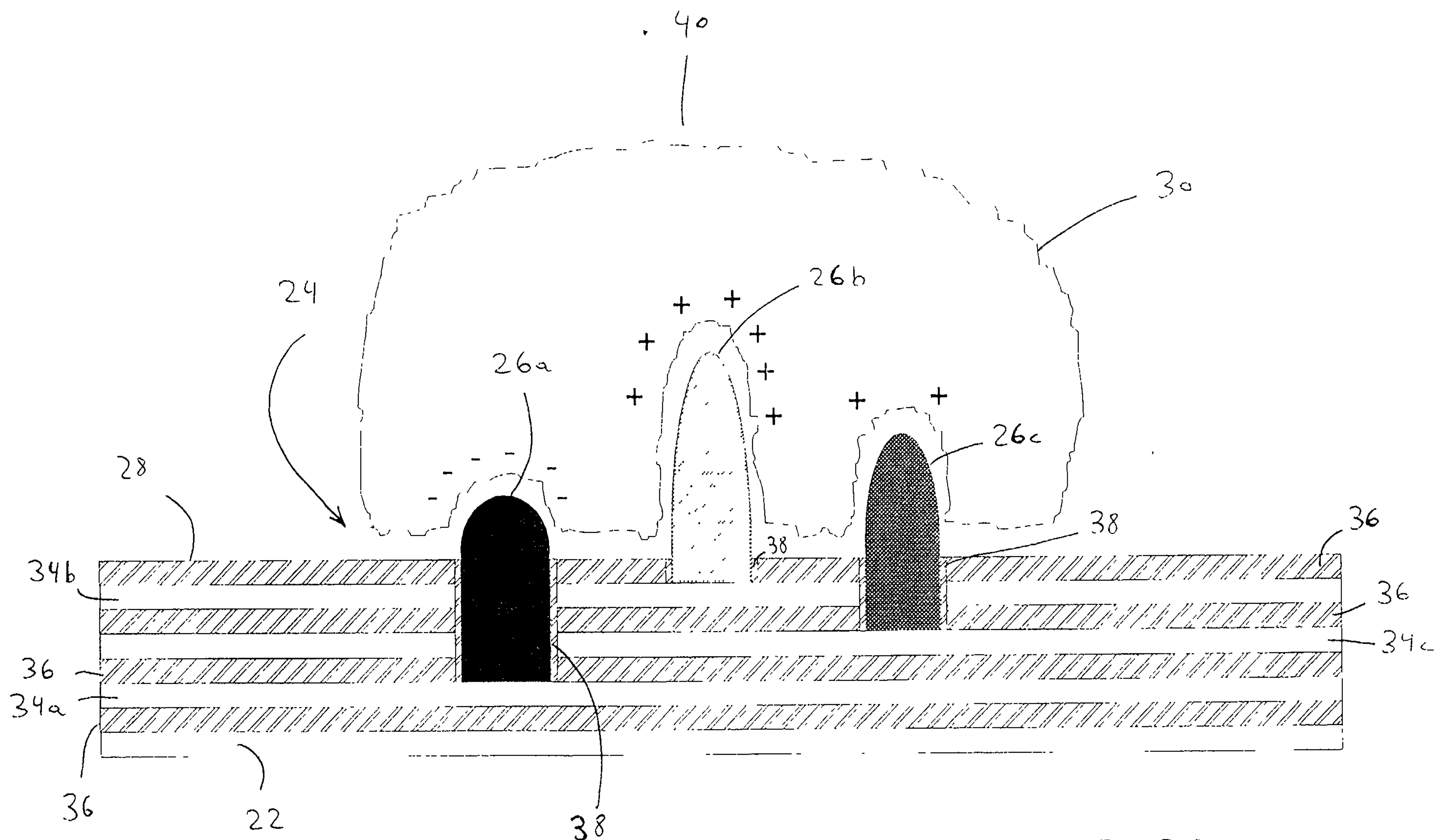

FIG. 2A

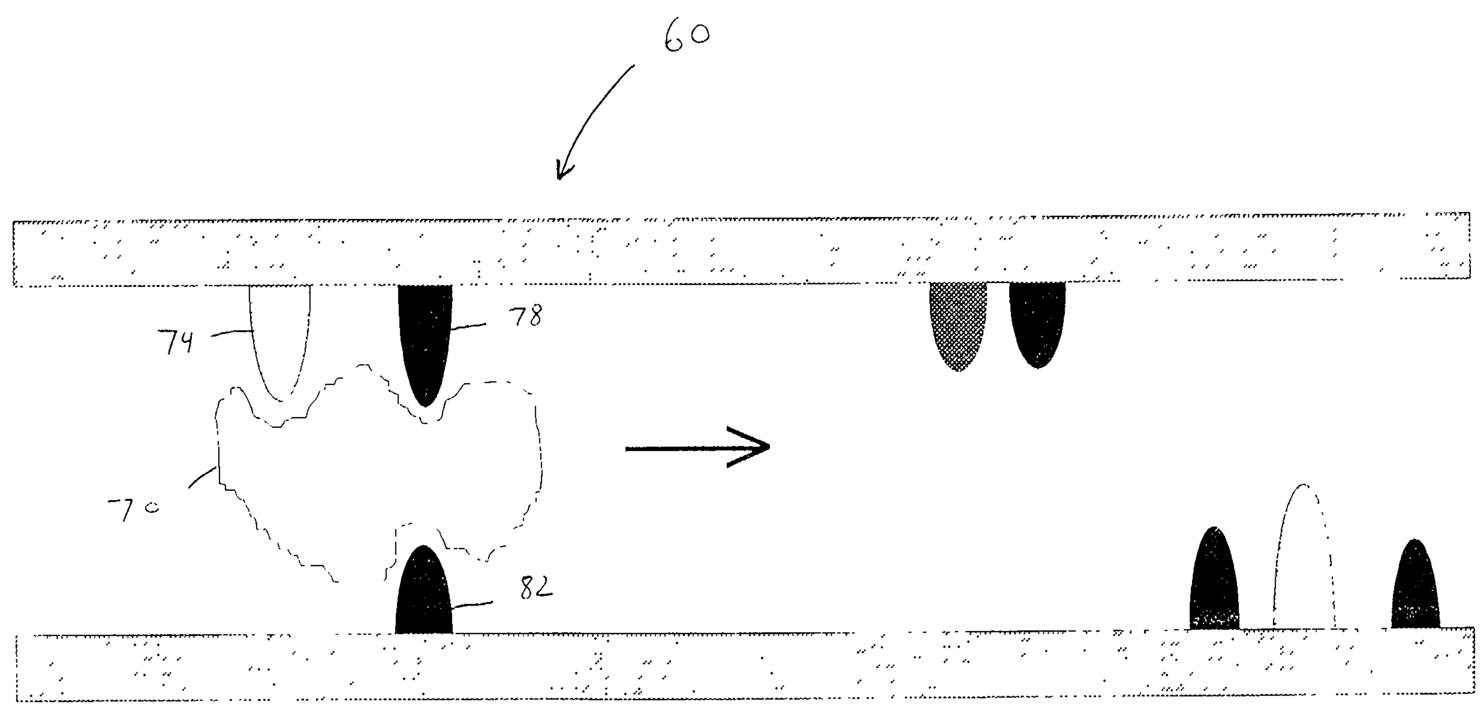

FIG. 3

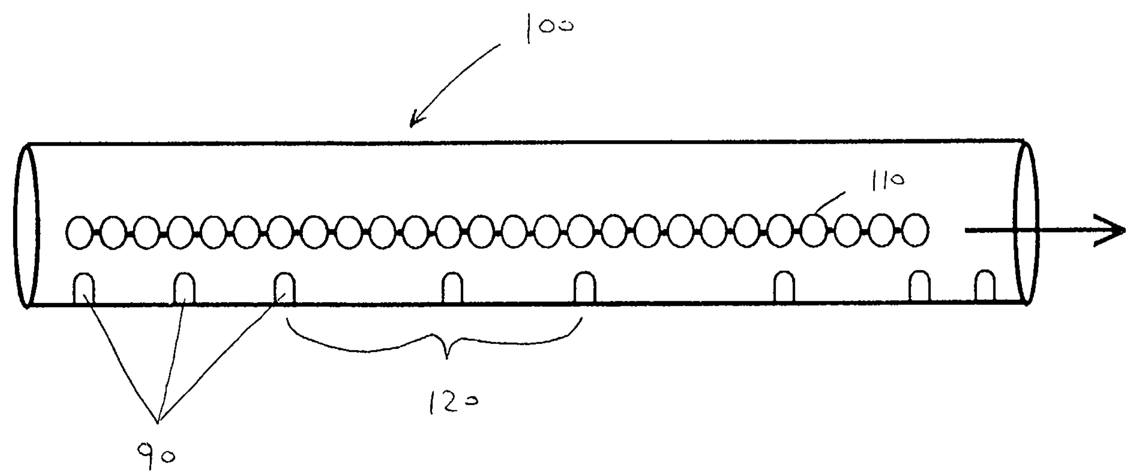

FIG. 4

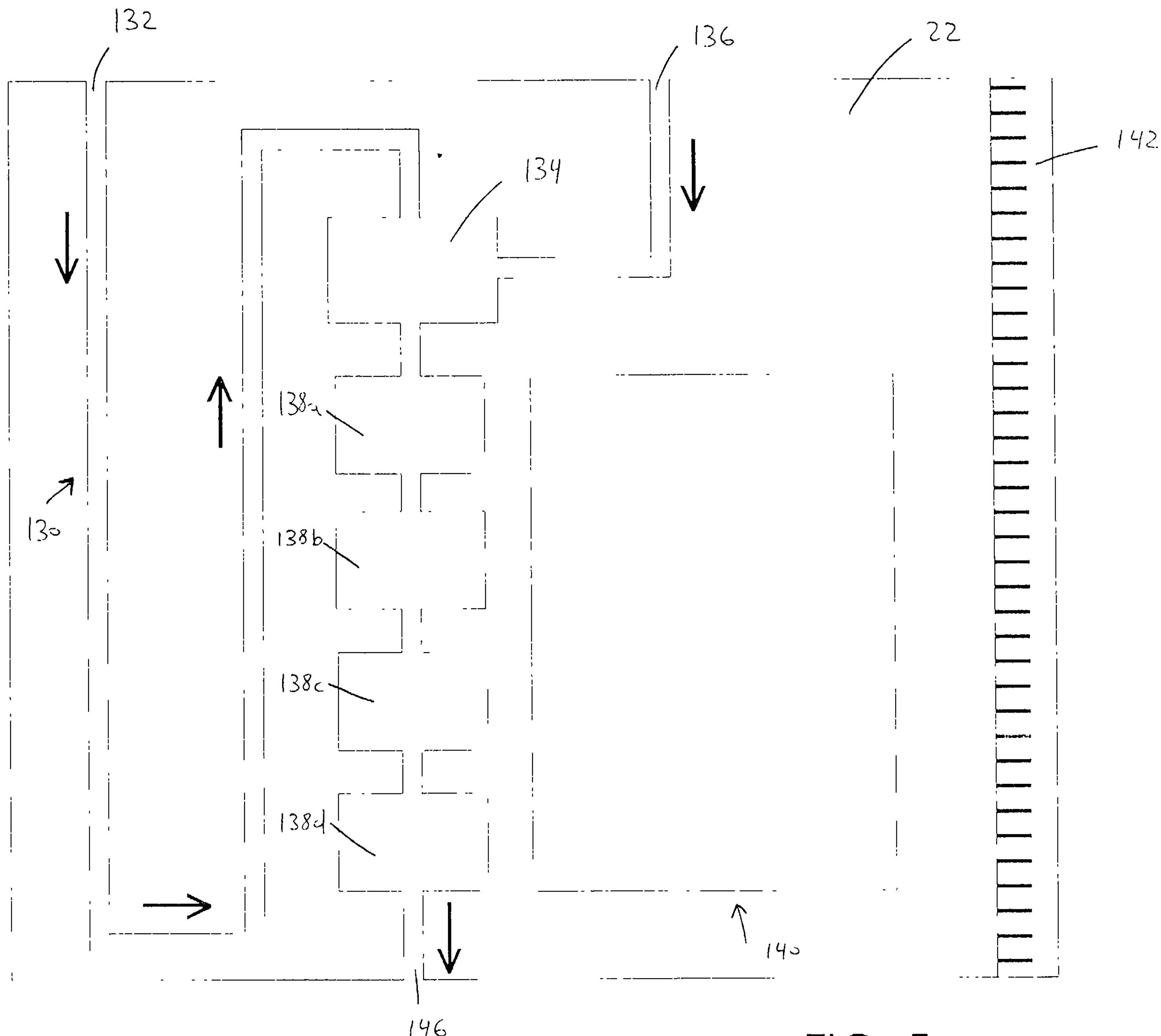

FIG. 5

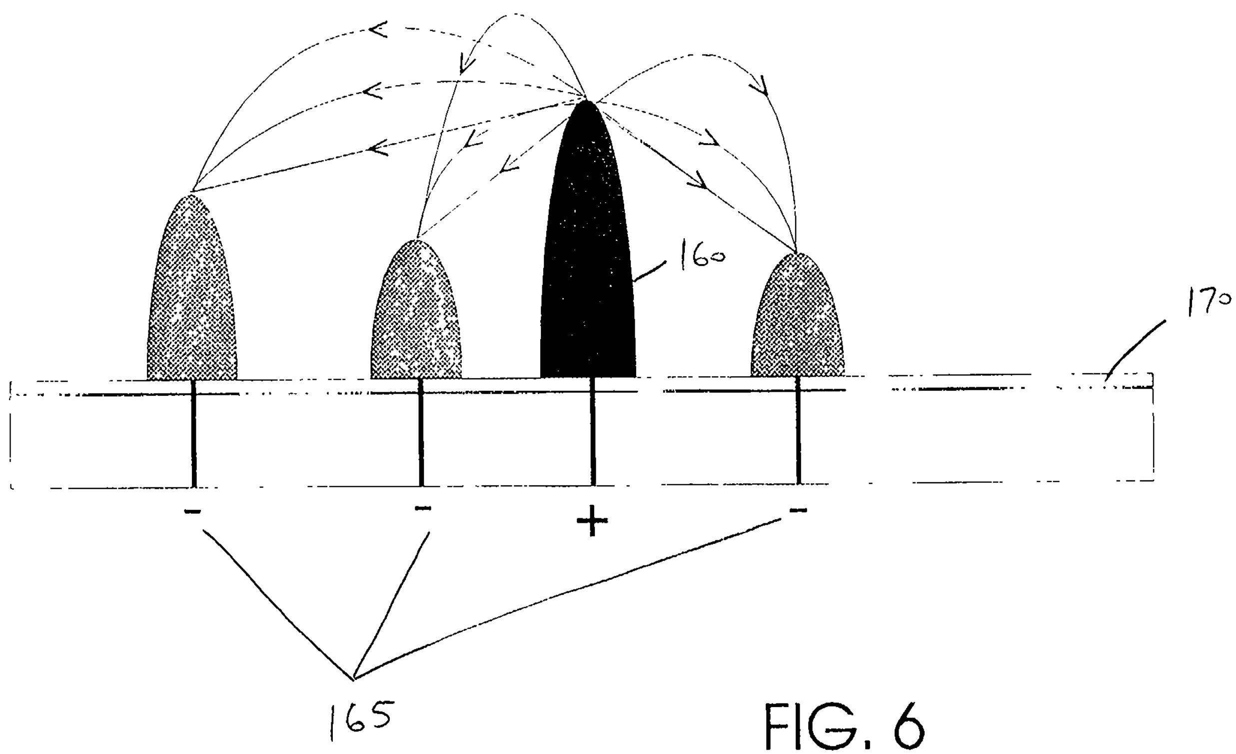

FIG. 6

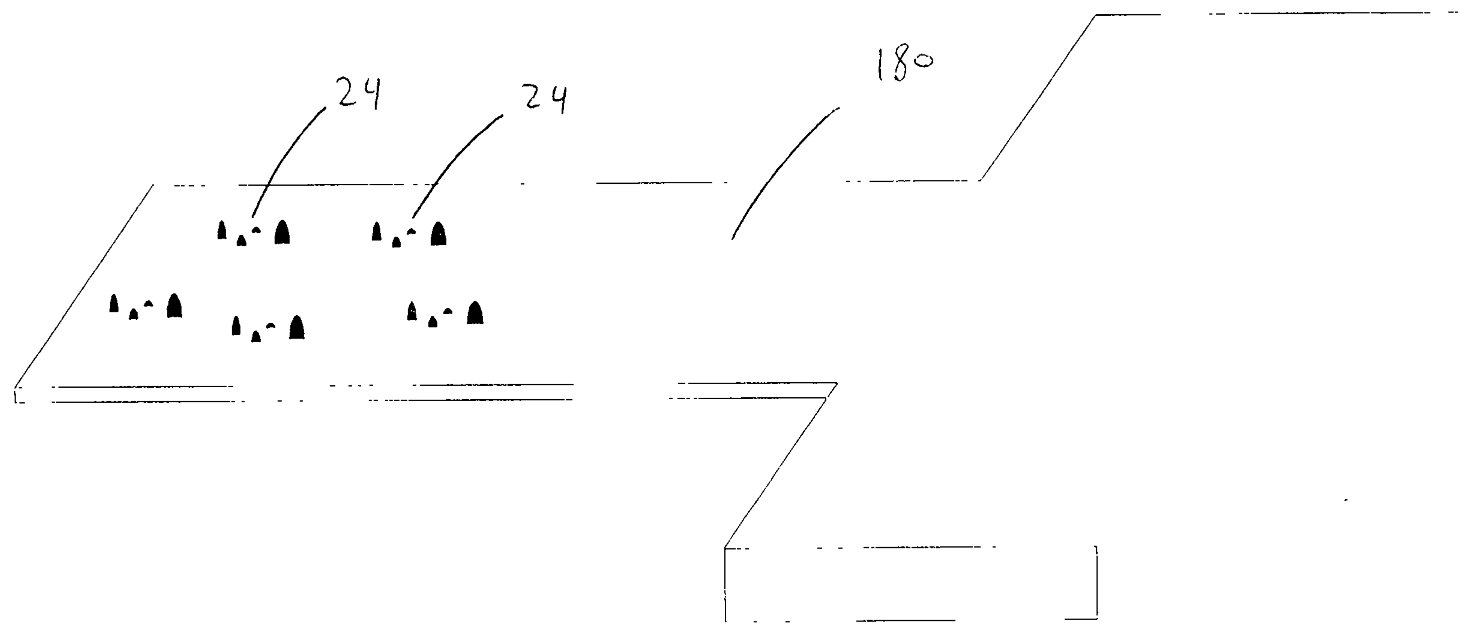

FIG. 7

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